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(54) Title: NEUROACTIVE PEPTIDES**(57) Abstract**

Compositions and methods are described for the specific identification of NMDA receptors, and for the specific identification and manipulation of NMDA glycine co-agonist biological activity.

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Title: Neuroactive Peptides**Related Applications**

This application claims priority to U.S. Patent Application Serial Number
5 08/649,272 filed 17 MAY 1996, the specification of which is hereby incorporated by
reference in its entirety.

Background of the InventionField of the Invention

10 The instant invention is related to the field of neuroactive peptides, proteins, or
amino acid compositions.

Description of the Related Art

It is now well known that the central nervous system (CNS) of mammals
15 employs many neuroactive peptides to effect specialized signaling within the brain and
spinal cord. Among the more well known neuroactive peptides are Somatostatin,
Cholecystokinin, VIP, Substance P, Enkephalin, Neuropeptide Y (NPY), Neurotensin,
TRH, CCK, and dynorphin. (*see generally The Biochemical Basis of
Neuropharmacology*, Cooper, Bloom and Roth, 5th ed., Oxford University Press, New
20 York, 1986). The careful elucidation of the complex signaling pathways which operate
in the CNS requires the identification and characterization of specific neuroactive
peptides and their particular properties, as well as the characterization and localization of
specific neurologically significant receptors. Identification of agonists and antagonists
of CNS receptors, whether partial, complete, coordinately acting, or independently
25 acting, is useful in that the more that is known of specific neuroactive peptides, the
greater the range of manipulations that can be conducted on CNS receptor proteins, and
the behavior of CNS receptor complexes. Significantly, the identification of unique
agonists or antagonists allows for the fine characterization and localization of subsets of
neuroactive receptors by their binding to these agonists or antagonists. By identifying
30 neuroactive peptides, and using them to specifically perturb the behavior of known
receptor complexes, more detailed understanding becomes available about the receptor
complex. In addition, new neuroactive peptides offer alternative means of altering the

behavior of known CNS receptor complexes, or for the discovery of previously unknown receptor complexes or unknown behavior of known receptors.

The N-methyl-D-aspartate (NMDA) receptor, which has been implicated in neurodegenerative disorders, stroke-related brain cell death, convulsive disorders, learning and memory, has been cloned from human tissue (see Hoffman, M., 1991, *Science*, **254**:801-2). In addition to being activated by binding to NMDA, the NMDA receptors are activated by glutamate (Glu), and aspartate (Asp), as well as being competitively antagonized by D-2-amino-5-phosphonovalerate (D-AP5; D-APV), or non-competitively antagonized by phenylcyclidine (PCP), and MK-801. However, most interestingly, the NMDA receptor is co-activated by glycine (Gly). (Kozikowski et al., 1990, *Journal of Medicinal Chemistry* **33**:1561-1571). The binding of glycine to an allosteric regulatory site on the NMDA receptor complex increases both the duration of channel open time, and most dramatically the frequency of the opening of the NMDA receptor channel.

The NMDA receptor is considered central to long-term potentiation (LTP), which is the persistent strengthening of neuronal connections that is considered to underlie learning and memory (see Bliss and Collingridge, 1993, *Nature* **361**: 31- 39, for review). Damage to the CNS, which may occur for example during a stroke, is thought to cause the over-excitement of cells which have the NMDA receptor by flooding of glutamate or aspartate, leading to the death of some 80% of such overexcited cells. The bulk of NMDA receptor carrying cells are in the cortex and hippocampus regions of the brain, and after such overexcited killing of cells, patients are rendered incapable of learning new things, but can still recall items in long term memory. Human memory deficits associated with PCP abuse have been linked to the action of PCP, and is an expected consequence of the inhibition of calcium fluxes through the NMDA receptor.

It is thought that drugs which can block, or otherwise alter the operation of the NMDA receptor may protect cells from overexcited killing, or NMDA receptor associated memory problems. Other drugs that interact with the NMDA receptor may enhance the ability of the cells to form LTP and thus enhance learning and memory.

Because of the significance of the NMDA receptor, it would be most useful to have specific peptide agonists or antagonists which will allow for fine mapping of the tissue distribution, subset characterization, and fine manipulation of NMDA receptors,

and for characterization of the action of other agonists or antagonists on the NMDA receptor.

Summary of the Invention

5 The instant invention provides certain specific neuroactive peptides which are characterized by the ability to bind to the NMDA receptor. The instant invention provides for specific polypeptides or amino acid compositions which bind to the NMDA receptor at the glycine co-agonist site and effect at least the same biological activity from the NMDA receptor as the binding of glycine. The instant invention provides for
10 polypeptides or amino acid compositions that are purified from natural tissues, fluids, or cells. The instant invention provides for the chemical synthesis of the polypeptides or amino acid compositions of the instant invention using conventional biochemistry methods, or molecular biology techniques. As the polypeptides or amino acid compositions of the instant invention are useful for the isolation and characterization of
15 NMDA receptor activity and tissue localization, the instant invention provides for stabilized polypeptides or amino acid compositions wherein the backbone has incorporated modified peptides such that stability is enhanced, or via addition of framework modifications such that the three-dimensional conformation of the peptide fragment is stabilized or enhanced. The instant invention also provides for cyclized
20 polypeptides or amino acid compositions.

An additional benefit of the polypeptides or amino acid compositions of the instant invention is that the small size will enhance the ability to cross the blood-brain barrier, and are thus suitable for *in vivo* administration and detection. Thus the instant invention encompasses polypeptides or amino acid compositions of the instant invention
25 coupled to radioactive markers, MRI markers, metal ion markers, enzymatic markers, chemiluminescent markers, or any such marker which will allow for the detection of the polypeptide or amino acid compositions. The instant invention also encompasses pharmaceutical formulations of the polypeptides or amino acid compositions of the instant invention, in suitable pharmaceutical carriers such that they can be administered
30 to a living subject. Such administration can be *i.p.*, *i.v.*, *i.m.*, or by any other appropriate means.

In instances where the polypeptides or amino acid compositions of the instant invention are used for detection of NMDA receptor using *in vitro* screening such as

tissue section and staining, the attached marker can also be, in addition to the suitable markers above, proteins, antibody, avidin, biotin, and any other such marker which allows for the detection of the presence of polypeptide or amino acid compositions in screening assays or staining procedures.

5 The instant invention also provides for methods of detecting NMDA receptor using the polypeptides or amino acid compositions of the instant invention and an appropriate marker for detection of the bound receptor and polypeptide or amino acid compositions. Such methods can be practiced *in vitro* and *in vivo* depending on the conditions for detection used. Certain procedures which can be employed using such
10 methods include, and are not limited to, MRI, CAT scan, X-ray, Sonogram, and other such non-invasive detection methodologies. Where invasive procedures are contemplated, i.e. biopsy or tissue section, the use of standard immunological screening methods can be used to detect the presence of bound receptor/polypeptide, or such binding can be specifically visualized via immunological staining or other such detection
15 means utilizing the wide range of available marker/detection systems. The instant invention therefore also provides for a method for modifying the biological activity of a NMDA receptor comprising said NMDA receptor contacting with a polypeptide or amino acid compositions of the instant invention. Specifically encompassed are peptides having the amino acid sequences as listed in Table 1 below, that are predicted to be
20 effective by assay data in the following examples.

Table 1

Name SEQ ID NO	Amino Acid Sequence
NT-1: SEQ ID. NO:1.	Lys-Ala-Ser-Gln-Asp-Val-Ser-Thr-Thr-Val-Ala
NT-2: SEQ ID. NO:2.	Ser-Ala-Ser-Tyr-Arg-Tyr-Thr
5 NT-3: SEQ ID. NO:3.	Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr
NT-4: SEQ ID. NO:4.	Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr
NT-5: SEQ ID. NO:5.	Glu-Asp-Leu-Ala-Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-
	Thr-Pro-Pro-Thr
10 NT-6: SEQ ID. NO:6.	Ser-Val-Gln-Ala-Glu-Leu-Asp-Leu-Ala-Val-Tyr-Tyr-Ser-
	Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr
NT-7: SEQ ID. NO:7.	Phe-Thr-Ile-Ser-Ser-Val-Gln-Ala-Glu-Leu-Asp-Leu-Ala-
	Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-
	Thr
15 NT-8: SEQ ID. NO:8.	Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Phe-Gly-Gly-Gly
NT-9: SEQ ID. NO:9.	Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Phe-Gly-Gly-Gly-
	Thr-Lys-Leu-Glu
NT-10: SEQ ID. NO:10	Cys-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Cys [S S]
20 NT-11: SEQ ID. NO:11	Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Ser
NT-12: SEQ ID. NO:12	Gln-Gln-His-Tyr-Ser
NT-13: SEQ ID. NO:13	Thr-Pro-Pro-Thr
NT-14: SEQ ID. NO:14	Thr-Pro-Pro
NT-15: SEQ ID. NO:15	Pro-Pro-Thr
25 NT-16: SEQ ID. NO:16	Pro-Pro
NT-17: SEQ ID. NO:17	Thr-Pro-Pro
NT-18: SEQ ID. NO:18	Thr

Brief Description of the Drawings

30 Figure 1 shows three graphs comparing results of testing for NMDA binding using a [³H]MK801 assay. Figure 1A shows activity of D-cycloserine, Figure 1B shows the activity of monoclonal antibody B6B21, and Figure 1C shows the activity of peptide NT-3. Results are plotted as concentration of test component vs. percentage of control

binding of [³H]MK801 in the presence of 7-chlorokynurenic acid, a selective glycine site agonist.

Figure 2 shows the results of testing peptide NT-13 as a partial agonist in pharmacological NMDA-specific function assays as measured by the [³H]MK801 binding assay.

Figure 3 shows the results of testing peptide NT-13 as a partial agonist in electrophysiological NMDA-specific function assay. Figure 3A shows the results of patch clamp measurements. Figure 3B-D demonstrate that peptide NT-13 has characteristics of a partial glycine agonist. Figure 3B shows that NT-13 mimics glycine, but is not as effective. Figure 3C shows that NT-13 inhibits the standard NMDA+glycine current. Figure 3D shows that NT-13 induced NMDA current is blocked by 7-chlorokynurenic acid, which is a selective glycine site antagonist.

Figure 4 shows the results of testing peptide NT-13 as a partial agonist in a behavioral NMDA-specific function assay compared with cerebral spinal fluid control (CSF) and mAb B6B21. Figure 4 shows that peptide NT-13 produced some cognitive enhancement in the Morris water maze task as measured by short latencies to swim to the submerged platform (Figure 4A), path lengths to the platform (Figure 4B), and decreased average distance to the target during the probe trial on Day 8 of training (Figure 4C).

Figure 5 shows the results of eye-blink response in aging rabbits after treatment with B6B21 monoclonal antibody (mAb). Inset shows data for acquisition as maximum Conditioned Responses (CRs) achieved.

Figure 6A is a graph showing relative blood-brain barrier permeability of radiolabeled peptide NT-13 in comparison with radiolabeled antipyrine, sucrose and water. Figure 6B graphs the % counts in brain relative to water (100 % value).

Figure 7 graphs the amount of radiolabeled NT-13 measured in the brain 5 minutes after trans-nasal administration. Data is plotted as μg of radiolabeled NT-13 in the brain against mg of radiolabeled NT-13 delivered trans-nasally.

Figure 8 graphs the amount of radiolabelled NT-13 measured in the brain 20 minutes after *i.p.* injection. Data is plotted as μg of radiolabeled NT-13 in the brain against mg of radiolabeled NT-13 delivered via *i.p.* injection.

Detailed Description of the Invention

Example 1 Monoclonal Antibody specific for NMDA receptor

Monoclonal antibodies were generated by immunizing mice with dentate gyrus tissue isolated from 5 day old neonatal rats following standard protocols (*see* Moskal and Schaffner, 1986, *The Journal of Neuroscience*, 6(7):2045-2053). After screening the hybridomas for binding to dentate gyrus tissue via histochemical methods, promising candidate clones were isolated. Among the isolated clones was monoclonal antibody B6E11 which was found to block the production of LTP in rat hippocampal slices, and to suppress established LTP in both area CA1 of the hippocampus and the dentate gyrus (*see* Stanton, Sarvey and Moskal, 1987, *Proceedings of the National Academy of Science, U.S.A.*, 84:1684-1688). The monoclonal antibody B6E11 was found to be effective in blocking LTP when applied to the apical dendrites synapsing with the potentiating input, but not when applied to cell bodies or to the basal dendrites of CA1. In contrast, a second monoclonal antibody, G6E3, which was from the same panel, of the same immunoglobulin class, and bound to the target tissue in a similar fashion, did not have any effect on LTP.

Another monoclonal antibody, B6B21, was found to enhance LTP by glycine-like modulation of the NMDA receptor (*see* Haring, Stanton, Scheideler, and Moskal, 1991, *Journal of Neurochemistry*, 57(1):323-332). This unique monoclonal antibody was able to significantly elevate LTP when applied to CA1 pyramidal cell apical dendrites in rat hippocampal slices as detected by an elevation in binding of N-(1-(2-thienyl)cyclohexyl)-3,4-[³H]piperidine ([³H]-TCP). This effect was eliminated by the maximal saturation of the NMDA receptors with combined addition of maximal concentrations of glutamate, glycine, and magnesium. Most importantly, monoclonal antibody B6B21 reversed 7-chlorokynurenic acid inhibition of [³H]-TCP binding, but had no effect on the inhibition of [³H]-TCP binding by APV. The enhancement of the binding of [³H]-TCP by monoclonal antibody B6B21 was increased by glutamate but not glycine.

Hippocampus-dependent learning was found to be facilitated by the binding of B6B21, or the addition of D-cycloserine, both of which bind specifically to the NMDA receptor, in *in vivo* experiments utilizing a rabbit eye-blink conditioning test (*see*

Thompson, Moskal, and Disterhoft, 1992, *Nature*, **359**:638-641). Intraventricular (into the brain ventricle) infusions of B6B21 significantly enhanced acquisition rates in hippocampus-dependent trace eye-blink conditioning in rabbits, halving the number of trails required to reach a criterion of 80% conditioned responses. Peripheral injections 5 of D-cycloserine, a partial agonist of the glycine site on the NMDA receptor which crosses the blood-brain barrier, also doubled the rabbits' learning rates.

Study of the monoclonal antibody B6B21 allowed us to generate a panel of polypeptides or amino acid compositions (Table 1) which allow for the mimicking of the activity of the mAb B6B21, and thus the glycine co-agonist effect.

10

Example 2 Pharmacological NMDA Specific Activity - [³H]MK801 Assay

The peptides of the instant invention are capable of specific binding to the mammalian NMDA receptor at the glycine co-agonist site. Remarkably, the peptides of the instant invention do not require the presence of a glycine (Gly, G) amino acid.

15

Because of the significant role the NMDA receptor plays in the mammalian brain, specific agonists are very useful for the fine mapping of NMDA receptor tissue distribution, and correlation with disease, injury, or other pharmacological effects. Specific small peptide agonists are particularly useful in that they can be further modified for enhanced bioavailability and for transport across the blood-brain barrier.

20

Polypeptides or amino acid compositions of the instant invention were tested for the ability to mimic the glycine co-agonist effect on the NMDA receptor using a previously validated [³H]MK-801 binding assay. This functional assay takes advantage of the fact that *increased* [³H]MK-801 binding by NMDA receptors can only occur upon receptor-binding channel opening. This is because the MK-801 binding site is located inside the ionophore of the NMDA receptor complex, and is thus only accessible upon the opening of the receptor complex channel. Thus increased binding of [³H]MK-801 is directly correlated with increased channel opening, in this assay, triggered by the binding of the polypeptides or amino acid compositions of the instant invention to the glycine co-agonist binding site.

25

To further refine the assay, a selective antagonist of the glycine binding site of the NMDA receptor-channel complex is added to the assay. The normal action of 7-chlorokynurenic acid is to selectively bind to the glycine site, and inhibit NMDA

receptor channel opening, and thus inhibit the binding of [³H]MK-801 to the NMDA receptor complex. The addition of peptide NT-13 reversed the inhibition of binding of [³H]MK-801, and thus correlates with the finding that the polypeptides or amino acid compositions of the instant invention bind to the glycine binding site.

5 *Membrane Preparation* Crude synaptic membranes used in the assay were prepared using rat hippocampal tissue (male Sprague-Dawley rats) and extensively washed using the procedures described previously (Haring et al., 1991, *J. Neurochem.* **57**:323-331). Briefly, tissue which has been stored at -80°C is homogenized in ice cold 5 mM Tris (pH 7.4) using a Brinkman Polytron® and then pelleted by centrifugation at 10 48,000g for 20 minutes. The resulting supernatant is discarded, and the membranes washed three times in cold buffer. Pellets are then resuspended in 5 mM EDTA, 15 mM Tris (pH 7.4), and incubated for one hour at 37°C. The membrane suspensions are then pelleted by centrifugation at 48,000g for 20 min and stored at -80°C until use in the assay.

15 *Receptor Binding Assay* Frozen pellets are thawed at room temperature and washed three times by resuspension in 5 mM Tris (pH 7.4) and centrifugation. Final pellets are suspended at concentrations of 2 to 3 mg/ml in 5 mM Tris buffer (pH 7.4). Binding reactions are initiated by the addition of 200µg of freshly prepared membranes to reaction mixtures (about 1 ml final volume) containing 1nM [³H]MK-801 at 25°C in 20 the presence of a range of peptide concentrations and 60µM 7-chlorokynurenic acid. Non-specific binding is determined using 10µM unlabelled MK-801. Binding reactions are terminated by filtration through a Brandel 24-well cell harvester onto Whatman GF/B glass filters that have been presoaked in 0.25% polyethyleneimine for 30 minutes.

25 *Data Analysis/Interpretation* Peptides which stimulate [³H]MK-801 binding at concentrations equal to or less than the effective dose of D-cycloserine (at 10⁻⁵) are deemed to be positive for ability to bind the glycine site of the NMDA receptor complex.

30 Figure 1 shows data comparing the binding data using D-cycloserine (Figure 1A), mAb B6B21 (Figure 1B), and peptide NT-3 (Figure 1C). Figure 1C clearly shows that peptide NT-3 clearly binds to the glycine site of the NMDA receptor in a fashion similar to mAb B6B21, and D-cycloserine. Data is reported as % control binding in the presence of 7-chlorokynurenic acid against concentration of tested material.

Figure 2 shows the binding activity of peptide NT-13 as above. Data is reported as % control binding in the presence of 7-chlorokynurenic acid against concentration of tested material.

Table 2 below lists results from assay results using the peptides of the instant
5 invention.

TABLE 2

	<u>name</u>	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
	NT-1*			97±2	100±2	73±6
10	NT-2*		80±9	109±15	106±4	77±12
	NT-3	112±20	123±0	121±4	111±11	90±1
	NT-4	106±2	106±6	116±2	110±1	109±10
	NT-5	109±8	100±9	114±2	106±2	112±4
	NT-6	95±6	101±9	101±15	96±1	91±7
15	NT-7	96±4	103±4	97±1	104±0	100±3
	NT-8	105±7	110±5	108±4	107±6	115±6
	NT-9	104±12	126±7	125±5	112±10	120±3
	NT-10	100±2	90±8	114±3	128±4	116±12
	NT-11	96±23	111±4	118±4	120±11	127±21
20	NT-12	95±21	104±10	95±7	106±9	106±2
	NT-13	97±8	103±8	110±10	129±10	117±7
	NT-14	89±6	101±1	96±5	98±8	95±4
	NT-15	99±21	103±2	100±7	108±12	93±1
	NT-16	83±9	89±8	97±6	103±3	117±12
25	NT-17	122±23	125±17	128±9	117±14	119±21
	NT-18	88±21	104±33	101±4	103±6	123±19

*In the presence of 10 μ M L-glutamate

Table 2 shows the effect of peptides NT-1 through NT-18 on NMDA receptor
30 activation as measured by [3 H]MK-801 binding in rat hippocampus assay, reported as a percentage of control binding (\pm S.E.M.) at various concentrations of peptide. The data reported for peptides NT-15 through NT-18 and the experimental error for each point

make it unlikely that any of these peptides have meaningful biological activity. It should be emphasized that peptide concentrations of 10^{-5} M, approaching millimolar range (100 mM), for efficacious concentration are too high to be considered reliable indicators that the peptide is biologically active. At such high concentration, it is likely that non-specific binding effects are magnified in relation to specific binding such that the binding equilibrium of the receptor/ligand is skewed. Thus, those peptides showing optimal binding activity in the 10^{-5} M concentration range are not considered likely candidates.

For example, NT-14 and NT-15 are without effect at any concentration tested, 10 and NT-16, NT-17 and NT-18 are most efficacious at 10^{-5} M. By taking into account the concentration and the experimental error, it is reasonable to exclude these peptides as not being biologically active.

However, it is noted that D-cycloserine is a partial agonist at the glycine site of the NMDA receptor, and can act as a cognitive enhancer (Thompson, Moskal, and 15 Disterhoft, 1992, *Nature*, **359**:638-641). Serine and threonine do share many structural features, such that it may be possible for threonine to have similar enhancing properties as serine. Thus it is possible to predict that replacing the threonine moieties of NT-3 and NT-13 with serine, will result in active peptides.

20 Example 3 Electrophysiological NMDA Specific Activity Assay

Direct electrophysiological measurement of the effects of the peptides of the instant invention on NMDA receptor function is a powerful, unambiguous, and relatively cost and time efficient screening method. Peptides of the instant invention, first selected as favorable via the [3 H]MK-801 binding assay, are then subjected to 25 electrophysiological screening.

Methods Electrophysiological recordings are made with two-electrode voltage-clamp techniques, and standard oocyte expression system preparations (see Leonard and Kelso, 1990, *Neuron* **4**:53-60; Kelso et al., 1992, *J. Physiology* **449**:705-718). Oocytes are isolated and injected with mRNA for mouse NMDA receptor subunits: 30 70nl of z1 ($\xi 1$) RNA is co-injected with an equal volume of e1 ($\epsilon 1$) RNA. After incubation for two days, recordings are made while clamping membrane potential at

-80mV. The standard recording solution contains 95mM NaCl, 2mM KCl, 3.8mM BaCl₂, and 5 mM HEPES. Mg²⁺ is omitted because it blocks NMDA currents at some potentials; Ca²⁺ is omitted because it can trigger the oocyte's endogenous Ca²⁺-dependent chloride current. Responses are evoked by the continuous perfusion of 3 - 5 ml of NMDA containing solutions. The recording chamber has a volume of about 400ml. In some experiments, 7-chlorokynurenic acid is added to the perfusion solution to demonstrate that the peptides or amino acid compositions tested are able to compete with, and act at the glycine binding site.

Data Analysis/Interpretation

Figure 3A shows that peptide NT-13 has a dose-dependent effect on NMDA currents. Increasing concentrations cause increased currents.

Figure 3B shows that peptide NT-13 enhances NMDA current, in the absence of glycine. Here, 100μM NMDA with 10μM glycine elicited a large current (about 284 nA, downward deflection of trace). In the next phase, 100μM NMDA with 100μM peptide NT-13 elicited a significant current of about 40% of the saturated glycine+NMDA response. In this same cell, NMDA alone showed only a negligible response (about 7nA).

Figure 3C shows that at high glycine concentrations, added peptide NT-13 reduces NMDA current, here a reduction to about 91% of the control response to NMDA and saturating glycine. This data further supports the conclusion that the peptide competes at the glycine binding site.

Figure 3D shows that in the absence of glycine, the effect of peptide NT-13 was blocked by the selective addition of the antagonist 7-chlorokynurenic acid.

25 Example 4 Behavioral NMDA Specific Activity Assay - Morris Water Maze

Morris water maze There are two types of hippocampus (HPC)-dependent associative learning; a temporal-dependent and a spatial-dependent. The Morris water maze (Morris, 1984, *J. Neurosci. Meth.* **11**:47-60; Brandeis et al., 1989, *Int. J. Neurosci.* **48**:29-69) is a well-established paradigm for measuring spatial-dependent learning, and thus complements other behavioral assays such as eye-blink studies which measure temporal learning. Both types of learning are affected by aging and are dependent on NMDA receptor activation (Morris, 1989, *J. Neurosci.* **9**:3040-3057).

Method of Approach Adult male Sprague-Dawley rats are used in all experiments. A total of 8 to 10 animals per group are used. The number of animals per group was selected upon power function analysis of previous data obtained in similar work. Based upon the outcome of these prior studies, realistic estimates of error variance for biochemical and behavioral endpoints were obtained. Using this information and establishing the value as $p < 0.05$, the sample size was selected which would provide acceptable levels of statistical power. Estimates were based upon suggestions presented in Keppel (Design and Analysis, Prentice Hall, NY, 1973). In all instances studies were designed to maximize the amount of information derived from a minimum number of animals.

Rats are implanted with stainless steel guide cannulae in both the left and right lateral ventricle of the brain. Behavioral testing begins after the rats have rested for two weeks. Fifteen minutes prior to each day's training session in the water maze task, described below, rats are infused with peptide at appropriate concentrations, or with artificial cerebrospinal fluid control vehicle (aCSF). Solutions are infused at a rate of about 1.0ml/min into the ventricles (*i.c.v.*) using a 30 gauge injection cannula connected by PE-10 tubing to a 10ml Hamilton syringe mounted in a CMA Instruments precision infusion pump. A total of about 3.0ml is infused into each ventricle. To promote diffusion, the injection cannula is left in place for a period of two minutes following infusion. Acute injection procedures are used so as to maintain a specific treatment-testing interval. A non-injected control group is included to assess the effects of the injection procedure itself on the performance.

Water Maze Task Fifteen minutes following *i.c.v.* injection, rats are tested in a Morris water maze task (MWM). This task is widely used to assess the behavioral properties and neurobiological substrates of spatial memory in rats. It has become popular since it is a rapidly acquired behavior, and is not motivated by food deprivation. Numerous studies have shown that performance of this task is dependent upon intact hippocampal and septohippocampal cholinergic circuitry. Task performance is disrupted by (i) surgical, or (ii) pharmacological disruption of the HPC or its cholinergic innervation, and by (iii) aging. It is accepted that this task provides a sensitive and useful behavioral assay for the functional integrity of the septohippocampal pathway and the HPC (Opello et al., 1993, *Physiol. Behav.* **54(6)**:1227-1233).

The standard MWM task requires rats to learn to swim to a submerged platform in a circular pool containing opaque water. Four equally spaced points around the edge of the pool are designated as start positions, and divide the pool into four equal quadrants. The submerged escape platform is located in one of the four quadrants

5 throughout training. Each training trial consists of placing the rat into the water at one of the four start positions. The rat is allowed to search for the submerged platform for up to 60 seconds, and is allowed to remain on the platform for 30 seconds. The rats are tested for two trials per day, for 15 consecutive days. Different start positions around the pool are presented in a random sequence. Latency to swim to the submerged platform serves

10 as the measure of acquisition. These testing parameters provide a task that is sensitive to both treatment-induced improvements and impairments of spatial memory. A second component of testing involves the introduction of probe trials after trials 2, 14, 26 and 38. During the probe trials the escape platform is removed and the rat's behavior is videotaped for 30 seconds. The following measures are derived by an automated video

15 tracking system: (i) percent of time spent swimming in the correct quadrant, (ii) average distance from the target during the probe trial, and (iii) swim speed. The use of this sequential probe trial procedure allows the assessment of different components of spatial learning; procedural memory, a form of memory not related to hippocampal functions, and declarative memory, a HPC-dependent process. It is important to evaluate these two

20 forms of cognition since only declarative memory is compromised in aging, Alzheimer's disease, and in the AF64A model (Opello et al., 1993, *Physiol. Behav.* **54(6)**:1227-1233).

Histological Analysis Following the completion of the behavioral studies, rats are sacrificed and prepared for histological examination. Analysis is done to determine if repeated injection of peptides produce any signs of excitotoxic damage in the HPC. Blocks of HPC will be drop fixed in a 0.1M phosphate buffer solution containing 10% formalin and 30% sucrose. Coronal sections are cut and stained with cresyl violet, staining of the pyramidal cell layers in CA3 and CA1 is measured with an image analysis system.

Data Analysis/Interpretation Overall treatment effects are assessed using

30 either a one or two way analysis of variance (ANOVA), depending on the occurrence of multiple factors or repeated measures, according to a mixed model ANOVA. Appropriate pair-wise comparisons are performed using Fisher's Least Significant

difference (LSD) test. Acceptable statistical significance is $p < 0.05$, and all post-hoc tests are two-tailed.

Results Figure 4 shows the results of testing peptide NT-13 as a partial agonist in a behavioral NMDA-specific function assay compared with cerebral spinal fluid control (CSF) and mAb B6B21. The results show that peptide NT-13 produced some cognitive enhancement in the Morris water maze task as measured by shorter latencies to swim to the submerged platform (Figure 4A), decreased path lengths to the platform (Figure 4B), and decreased average distance to the target during the probe trial on Day 8 of training (Figure 4C). The data in Figure 4A are presented as Escape Latency 5 in seconds for each tested treatment. Here the rats treated with peptide NT-13 showed markedly improved escape latency time (about 10.75 seconds) as compared with CSF control (about 19 seconds), and mAb B6B21 treated animals (about 16.25 seconds). The data in Figure 4B are presented as Path Length in centimeters for each tested treatment, where rats treated with peptide NT-13 showed decreased path length to target (about 300 10 cm); as compared to control CSF treated (about 420 cm) and mAb B6B21 treated animals (about 330 cm). The data in Figure 4C are presented as Distance to Target in centimeters for each tested treatment, where the average distance from the target, once removed during the probe trial, is used as a measure for retention. Here rats treated with peptide NT-13 stayed very close to the location of the removed target, an average 15 distance of about 31 cm, while control CSF and mAb B6B21 treated animals strayed about 36.25 cm and 35 cm, respectively.

20

These data demonstrate that treatment with the peptide of the instant invention can induce *in vivo* behavioral effects in mammals, specifically cognitive enhancement as demonstrated by improved performance in the Morris water maze task.

25

Example 5 Behavioral NMDA Specific Activity Assay - Trace Eye-blink

Trace Eye-blink Conditioning Eye-blink or nictitating membrane conditioning has been adapted as a "model behavioral system" for use in the analysis of neural substrates of learning by several laboratories (Disterhoft et al., 1977, *Brain Res.* 30 137:127-143; Thompson, 1976, *American Psychologist* 31(3):209-227). Among the advantages of this system are the relative simplicity of the behavioral paradigm, the excellent control procedures available, the fact that associative learning is being analyzed, the ease of conditioned and unconditioned stimulus application and control,

the ease of precise behavioral and neurophysiological measurement, and the extensive body of behavioral data which are available for this preparation (Gormezano, 1966, in *Classical Conditioning*, J.B. Sidowdki ed., McGraw Hill, New York, pp.385-420; Gormezano et al., 1987, *Classical Conditioning*, Hillsdale, New Jersey). The system has
5 been correlated with several pathologies, including memory disorders related to aging (Solomon et al., 1988, *Neurobiol. Aging* 9:535-546), calcium deficiency and aging (Disterhoft et al., 1994, *Annals NY Acad. Sci.* 747:382-406), amnesia (Gabrieli et al., 1995, *Behav. Neurosci.* 109:819-827), and amnesic Korsakoff's patients and recovered alcoholics (McGlinchey-Berroth et al., 1995, *Alcoholism: Clin. and Exp. Resch.* 19:1127-1132).

10 *Method of Approach* Female adult albino rabbits, *Oryctolagus cuniculus* were surgically implanted with lateral ventricular guide cannulae bilaterally and fitted with restraining headbolts. Surgery was performed at least one week after arrival, and dosages for anesthesia were calculated according to weight (60 mg/kg
15 ketamine-HCl, 10 mg/kg xylazine).

Approximately 10 days after surgery subjects were given a single, one hour session of habituation to the training environment. Training began after two days of rest. Rabbits were restrained using snug bags with drawstrings at the front and rear and trained in separate sound attenuated chambers. The rabbits were placed in a padded
20 plexiglass stock similar to that described by Gormezano et al., (1966, in *Classical Conditioning*, J.B. Sidowdiki ed., McGraw Hill, New York, pp.385-420), with a bar attached for head restraint. The eyelids were held open with dress hooks and nictitating membrane extension was measured with an infrared reflective sensor (Thompson et al., 1994, *J. Neurosci. Meth.*, 54:109-117).

25 Prior to each training session, rabbit pairs received bilateral infusions of 5 µl of either B6B21 suspended in artificial cerebral spinal fluid (aCSF; 124 mM NaCl, 26 mM NaHCO₃, 3 mM KCl, 2.4 mM CaCl₂, 1.3 mM MgSO₄, 1.24 mM NaH₂O₄, 10 mM D-glucose; pH 7.4), or of aCSF alone at a rate of 1 µl/min/ventricle. Three concentrations
30 of B6B21 were used; 0.3 µg/µl, 1.0 µg/µl or 3.0 µg/µl. The person conducting the experiment was blind as to the contents of the administered solution. Cannulated rabbits were trained in pairs counterbalanced among the four treatment groups with a maximum of six animals in each group.

Trace nictitating membrane conditioning began immediately after infusion. Training was given for 15 days with 80 trials/day (CS: 6 kHz, 90 dB, 100 msec, 5 msec rise/fall time; UCS: 3.5 psi tone, 150 msec). The trace interval was 500 msec to make the task dependent upon the hippocampus (Moyer et al., 1990, *Behav. Neurosci.* 104(2):243-252). Trials were presented with a variable 30-60 sec intertrial interval and controlled by an IBM PC-compatible computer system (Akase et al., 1994, *J. Neurosci. Method.* 54:119-130; Thompson et al., 1994, *J. Neurosci. Meth.*, 54:109-117).

Data Analysis/Interpretation Overall treatment effects are assessed using either a one or two way analysis of variance (ANOVA), depending on the occurrence of multiple factors or repeated measures, according to a mixed model ANOVA. Appropriate pair-wise comparisons are performed using Fisher's Least Significant difference (LSD) test. Acceptable statistical significance is $p < 0.05$, and all post-hoc tests are two-tailed.

Results In order to compare behavioral measures based upon the dosages of antibody B6B21 received, rabbits were grouped according to total amount of B6B21 received each day; a CSF control, 1.5 μ g, 3.0-5.0 μ g, and 10.0-15.0 μ g. The 1.5 μ g B6B21 drug group was not included in the statistical analyses because it consisted of only one subject.

The final results showed that B6B21 administration enhanced acquisition of the trace conditioned eye-blink response in aging rabbits in a dose dependent manner (Figure 5). Using an identical trace conditioning protocol, Thompson et al., (1995, *Neurobiol. Aging* 747:382-406) reported that 40% of 36+ month-old rabbits ($n=50$) reached a criteria of 80% CRs within 25 days of training. The remaining 60% of the rabbits failed to perform at a level $>30\%$, and were referred to as "severely impaired." The aCSF controls ($n=2$) in the present study performed in a similar fashion to Thompson's "severely impaired" animals, as did the one animal receiving 1.5 μ g B6B21 (Low Dose; $n=1$, data not shown). None of the rabbits in the present study, who received 10.0-15.0 μ g B6B21 (High Dose; $n=3$) reached 80% CRs within 15 days. However, rabbits receiving 3.0-5.0 μ g B6B21 (Intermediate Dose; $n=4$) began to show greater acquisition on Day 5 as compared to all other groups, and showed greater acquisition than controls as measured by the maximum CRs achieved (Student t-test: $t(4)=3.34$, $p < 0.05$, see Figure 5 inset).

The average percentage of CRs during the final week of training was generated for each group to measure learning. A one-way ANOVA between control and experimental groups indicated significant differences between group means ($F(3,12)=3.9$, $p < 0.05$ $p=0.037$). A Fisher's PLST post-hoc T-test comparison between 5 control and 3.0-5.0 μ g groups showed enhanced learning ($P(T \leq t)$ one-tail = 0.03, $p < 0.05$), whereas the higher dosage group did not show a significant difference in acquisition in the same post-hoc t-test ($P(T \leq t)$ one-tail = 0.09).

While the overall number of animals used for statistical analyses is small, these results clearly demonstrate that mAb B6B21 significantly enhances the acquisition of 10 trace eye-blink conditioning in aging rabbits in a dose-dependent fashion. These results show that there is reasonably expected success in developing biologically active B6B21 peptide mimetics. The hybridoma cell line producing the monoclonal antibody B6B21 has been deposited under the Budapest Treaty with the American Type Culture Collection (ATCC, Bethesda, MD, USA), under the ascension number _____ on 15 _____.

Example 6 *In Vivo Administration*

Permeability of the Blood-brain Barrier - Experiments were performed to determine the permeability of the blood-brain barrier to the peptides of the present 20 invention. The assay involved measurements 15 seconds after internal carotid injection *in vivo* of peptide of the invention, followed by detection in rat brain, and reported as a percentage of radiolabeled compounds administered. A mixture of labeled test substance and a standard reference substance was injected into to the rat common carotid with decapitation 15 seconds later. The ratio of [^{14}C] to [^3H] in brain tissue, relative to the 25 ratio of ^{14}C to ^3H in the original mixture administered, determined the amount of test substance lost to brain tissue on a single passage through the microcirculation of the brain. Because the data obtained is a ratio, the measurement is an accurate assessment independent of the quantity of test sample recovered. The test mixtures combined labeled ^{14}C -antipyrine or ^{14}C -sucrose with ^3H -peptide NT-13 or ^3H -Water as a control. 30 ^{14}C -antipyrine and ^{14}C -sucrose are diffusible and non-diffusible controls respectively (Figure 6A). Data was normalized using ^3H -Water as the standard reference substance (100%, Figure 6B). Additional data in Figure 6B reported for nicotine, isopropanol, heroin and codeine are taken from Bradbury et al., 1975, Amer. J. Physiol. 299:1110.

Transnasal Administration - Administration of the peptides of the instant invention can occur via the nasal pathway in aerosolized compositions or drops.

Experiments were performed to demonstrate the effectiveness of transfer to the brain after transnasal application of the peptides of the instant invention. Male Sprague-

5 Dawley rats were anesthetized with ketamine hydrochloride. Peptide solutions were a mixture of 0.05-0.1 mg of ^3H -NT-13 and a range of cold (unlabeled) NT-13 concentrations solubilized in Ringer's buffer (pH about 7.8). Dosing solutions were administered with a pipetteman to a single nare in two 20 μl doses. The second dose was delivered 2 minutes after the first, and the animal was decapitated 3 minutes later. Brains
10 were removed and quartered for solubilization. Once solubilized, samples were prepared for liquid scintillation counting. The data is reported in Figure 7, which graphs μg of labeled NT-13 measured in the brain against mg of labeled NT-13 administered transnasally.

15 *I.P. Injection of Peptides* - Experiments were performed to determine the amount of peptide delivered to the brain after *i.p.* injection of labeled peptides of the invention. Male Sprague-Dawley rats were injected with ^3H -NT-13 dissolved in water. Animals were then sacrificed 20 minutes after injection and the brains solubilized for liquid scintillation counting. Figure 8 is a graph showing the results graphing μg of ^3H -NT-13 measured in the brain against mg ^3H -NT-13 injected *i.p.*

20 As these experiments demonstrate, *in vivo* administration of the peptides of the instant invention can be accomplished effectively through a variety of routes.

Example 7 Hypothetical Approach

Behavioral NMDA Specific Activity Assay - Trace Eye-blink in Rats

25 *Method of Approach* The F1 hybrid of Fisher 344xBrown Norway rats are used because of minimal age-related pathology (Bronson, 1990, in *Genetic Effects on Aging II*, Harrison, D.E. ed., Telford Press, Caldwell, New Jersey) and age-related impairments in eye-blink conditioning (Weiss and Thompson, 1992, *Neurobiol. Aging* 13:319-323). Nine month old, virgin male rats are used in the experiments.

30 Rats are anesthetized with an intraperitoneal injection of sodium pentobarbital (65mg/kg body weight). The top of the head is shaved and cleaned with alcohol and betadine. A stereotaxic device with a gas anesthesia adapter and atraumatic ear bars (to protect the ear drums) are used. Once the animal is carefully secured, a midline incision

is made on the scalp. The skin of the pereosteum is retracted and the skull cleaned and dried. A hole is drilled through the skull approximately 0.8mm behind the bregma, and 1.3mm to the right and left of the midline (level head coordinates). The dura is then pierced, and a 25-gauge cannula is lowered into each hole to a depth of 4.0mm below the cortical surface (Tonkiss and Rawlins, 1991, *Exp. Brain Res.* **85**:349-358). These guide cannulae are then cemented to the skull with dental acrylic. A strip connector is cemented to the skull anterior to the cannula. The connector contains a ground wire, two wires (teflon™ coated stainless steel) which are implanted subdermally within the upper eyelid to measure EMG activity, and two wires which are implanted to deliver a periorbital shock. Subjects are given one week of recovery before habituation.

Fifteen minutes prior to each days training session, described below, rats are infused with peptide at appropriate concentrations, or with artificial cerebrospinal fluid control vehicle (aCSF). Solutions are infused at a rate of about 1.0ml/min into the ventricles (*i.c.v.*) using a 30 gauge injection cannula connected by PE-10 tubing to a 10ml Hamilton syringe mounted in a CMA Instruments precision infusion pump. A total of about 3.0ml is infused into each ventricle. To promote diffusion, the injection cannula is left in place for a period of two minutes following infusion. Acute injection procedures are used so as to maintain a specific treatment-testing interval. A non-injected control group is included to assess the effects of the injection procedure itself on the performance.

Rats are placed in a small cage in a sound attenuated chamber that has a speaker and ventilation fan. A cable is then connected between the experimental equipment and the strip connector implanted on the head. The conditioning stimuli is controlled by software running on a PC compatible computer and electronic modules from Coulbourn Instruments (Akase et al., 1994, *J. Neurosci. Meth.* **54**:119-130). The EMG activity is amplified, filtered, and full wave rectified with a time constant of 45 ms (Skelton, 1988, *Behav. Neurosci.* **102**:586-590). The signal is sent to a computer for data collection and analysis (Thompson et al., 1994, *J. Neurosci. Meth.* **54**:109-117).

Eye-blink conditioning is done using modified procedures as reported by Weiss and Thompson (1992, *Neurobiol. Aging* **13**:319-323). The rats are habituated to the conditioning apparatus for one 45 minute session prior to training sessions. Animals are trained daily in pairs for 15 days with either trace 500 paradigm or the unpaired control paradigm for psuedoconditioning. Rats are trace eye-blink conditioned using a tone

conditioning stimulus (CS, 100ms, 1KHz, 85dB, 5ms rise/fall time) and a periorbital shock unconditioned stimulus (US, 150ms, 2mA AC). The stimulus free trace period is 500 ms to make the task dependent upon the hippocampus (Moyer et al., 1990, *Behav. Neurosci.* **104**(2):243-252). Conditioned rats receive 80 trials with paired tones and 5 shocks at a random interval (ITI) of 30 - 60 seconds. Control rats receive 160 trials with either a tone alone, or a shock alone, at a random ITI of 15 - 30 seconds. After the conditioning sessions each rat undergoes five days of extinction training consisting of 80 sessions of tone alone trials with a 30 - 60 second ITI.

Histological Analysis Following the completion of the behavioral studies, 10 rats are sacrificed and prepared for histological examination. Analysis is done to determine if repeated injections of peptide produce any signs of excitotoxic damage in the HPC. Blocks of HPC will be drop fixed in a 0.1M phosphate buffer solution containing 10% formalin and 30% sucrose. Coronal sections are cut and stained with cresyl violet. Staining of the pyramidal cell layers in CA3 and CA1 is measured with an 15 image analysis system.

Data Analysis/Interpretation The data is analyzed with ANOVAs (one or two way analysis of variance) of group (conditioned vs. controls) x dose (ACSF and 3 doses). The repeated measures will also be analyzed with ANOVAs. The ANOVA for acquisition will include 15 levels in the ANOVA. The ANOVA for extinction will 20 include 5 levels. This factorial design yields 8 groups of animals with about 10 animals per group used for reliable statistical analysis.

Example 8 Future Autoradiography Studies

The peptides of the instant invention allow for the exquisite study of the brain 25 tissue distribution of biologically active peptide.

Method of Study By adapting the autoradiographic methods of Bekenstein et al., (1990) to the peptides of the instant invention, we will be able to examine the regional binding specificity throughout the hippocampal region of the brain, as well as other tissues. In the initial studies, optimized binding conditions will be developed in 30 terms of preincubations and washings, methods of drying, equilibrium of time course, saturation, and pharmacology of the binding site at 23°C. Naive rats will be sacrificed, the brains rapidly removed, embedded in OCT, and frozen in 2-methyl butane at -20°C prior to sectioning. Ten µm coronal sections will be cut on a cryostat and thaw-mounted

on poly-D-lysine coated coverslips. Serial sectioning will permit assessment of binding gradients along the entire septo-temporal and dorsal-ventral extent of the hippocampus. In the event that gradients in binding within (rather than across) specific hippocampal subfields or cell populations are found, we will take particular care to perform Scatchard analyses only within areas free from such gradients. Adjacent sections will be used for Nissel stained histological examination and for determination of nonspecific binding. Individual sections will be preincubated in several repeated volumes of 20 mM HEPES buffer (pH. 7.4) to reduce concentrations of endogenous or exogenous ligands. Tissue will be incubated with an optimized concentration of [³H]-peptide of the instant invention, in 20 mM HEPES buffer for varying periods of time, to determine equilibrium binding for saturation studies. For saturation experiments, brain sections will be incubated with varying concentrations of [³H]-peptide of the instant invention, in 20 mM HEPES buffer. Nonspecific binding will be determined with the addition of excess cold peptide.

15 *Analysis/Interpretation* Tissue sections will be apposed to [³H]-Ultrofilm (Amersham) and coexposed with methacrylate embedded tritium standards as needed for linear exposure of the film. A BioRad Phosphorimage Analyser will be used for these studies. Quantitative densitometric analysis will be performed on a Macintosh IIfx workstation with an 8-bit grey scale scanner, and public domain image analysis software 20 (ImageTM v. 1.29) developed at NIMH.

It will be understood that the specification and examples are illustrative, and not meant by way of limitation. One of ordinary skill in the art will be able to understand and determine from the teaching of the instant invention that other specific embodiments may be within the spirit and scope of the invention.

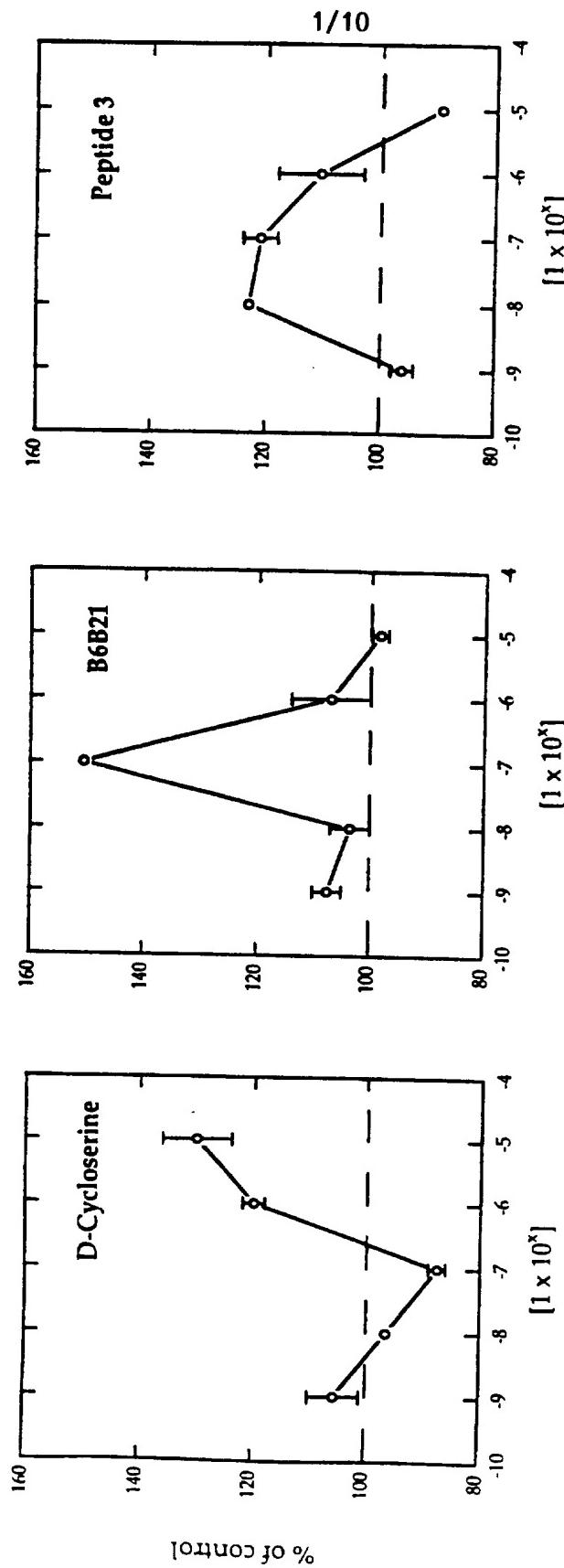
We Claim:

1. An isolated polypeptide having an amino acid sequence selected from the group consisting of:
Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:3); Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:4); Glu-Asp-Leu-Ala-Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:5); Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Phe-Gly-Gly-Gly-Thr-Lys-Leu-Glu (SEQ ID. NO:9);
Cys-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Cys (SEQ ID. NO:10);
[S S]
Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Ser (SEQ ID. NO:11); Gln-Gln-His-Tyr-Ser (SEQ ID. NO:12); and Thr-Pro-Pro-Thr (SEQ ID. NO:13).
2. A pharmaceutical composition comprising an effective NMDA receptor binding amount of an isolated polypeptide of Claim 1.
3. A method for detecting the presence of NMDA receptor in tissue comprising contacting a tissue to be tested with a labeled polypeptide of Claim 1, detecting the specific binding of said labeled polypeptide to tissue.
4. A method for modifying the biological activity of a NMDA receptor comprising contacting said NMDA receptor with a polypeptide of Claim 2 or pharmaceutical composition of Claim 2.
5. A pharmaceutical composition comprising an effective NMDA receptor binding amount of a polypeptide, at a concentration of from at least 10^{-5} M to the limit of solubility of the polypeptide, wherein said polypeptide is selected from the group consisting of:
Lys-Ala-Ser-Gln-Asp-Val-Ser-Thr-Thr-Val-Ala (SEQ ID. NO:1); Ser-Ala-Ser-Tyr-Arg-Tyr-Thr (SEQ ID. NO:2); Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:3); Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:4); Glu-Asp-Leu-Ala-Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:5); Ser-Val-Gln-Ala-Glu-Leu-Asp-Leu-Ala-Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr

(SEQ ID. NO:6); Phe-Thr-Ile-Ser-Ser-Val-Gln-Ala-Glu-Leu-Asp-Leu-Ala-Val-Tyr-Tyr-Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr (SEQ ID. NO:7); Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Phe-Gly-Gly-Gly (SEQ ID. NO:8); Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Phe-Gly-Gly-Gly-Thr-Lys-Leu-Glu (SEQ ID. NO:9);

Cys-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Cys (SEQ ID. NO:10);

Ser-Gln-Gln-His-Tyr-Ser-Thr-Pro-Pro-Thr-Ser (SEQ ID. NO:11); Gln-Gln-His-Tyr-Ser (SEQ ID. NO:12); Thr-Pro-Pro-Thr (SEQ ID. NO:13); Thr-Pro-Pro (SEQ ID. NO:14); Pro-Pro-Thr (SEQ ID. NO:15); Pro-Pro (SEQ ID. NO:16); Thr-Pro-Thr (SEQ ID. NO:17); and Thr (SEQ ID. NO:18).



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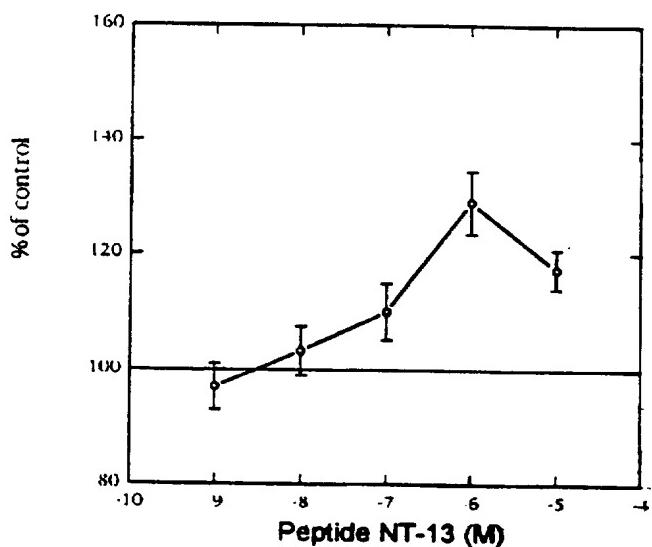


Figure 2

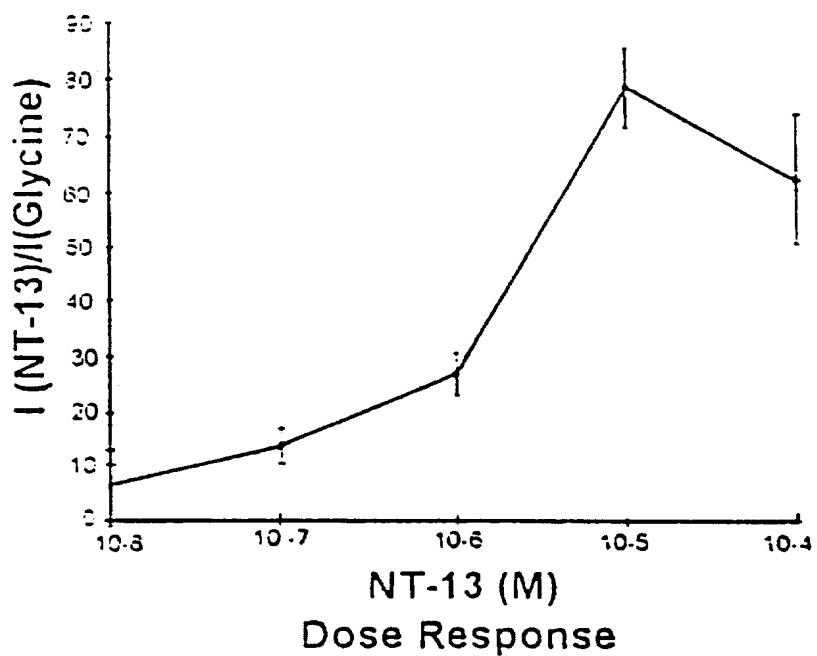
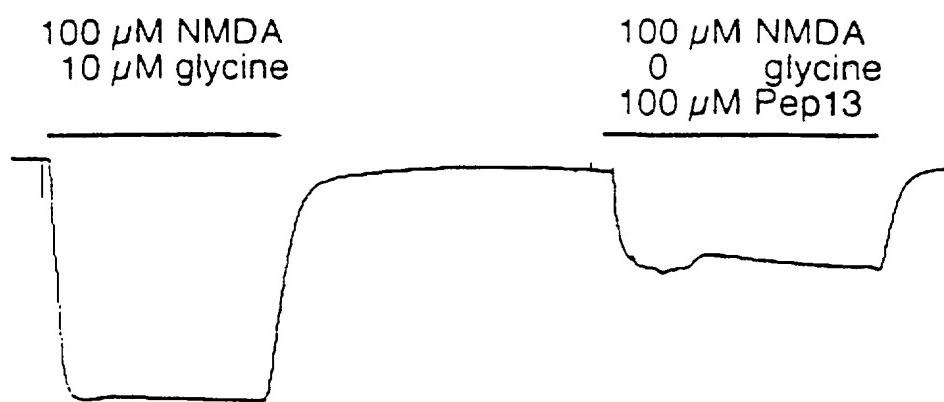
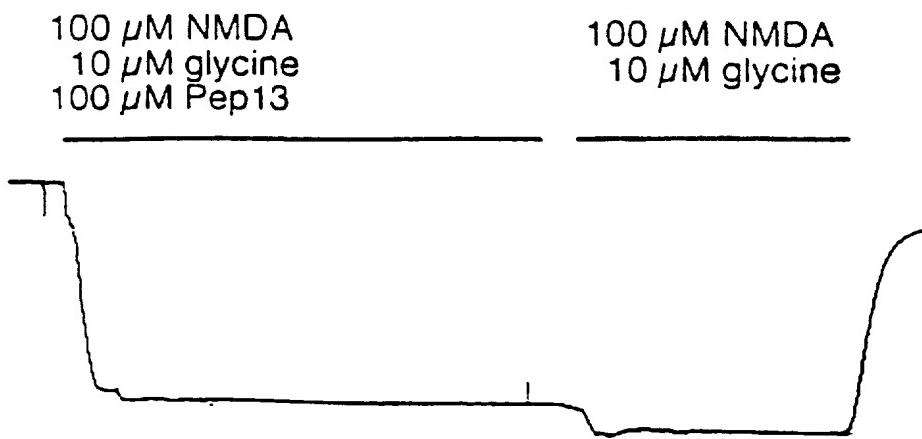
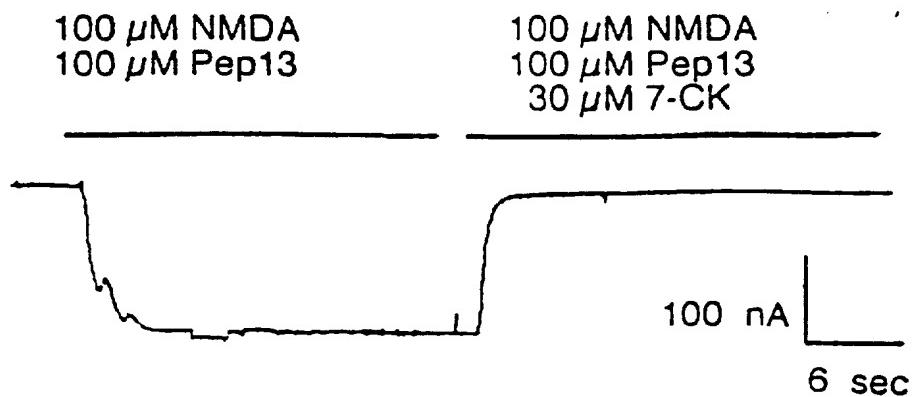


Figure 3A

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Figure 3B**Figure 3C****Figure 3D**

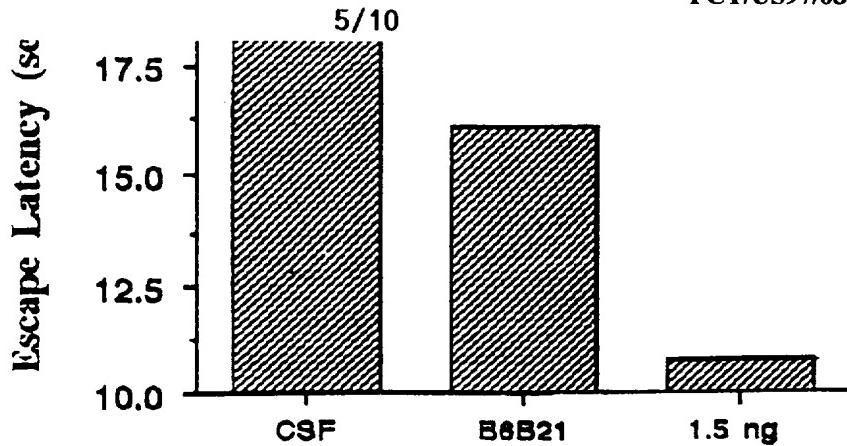


Figure 4A

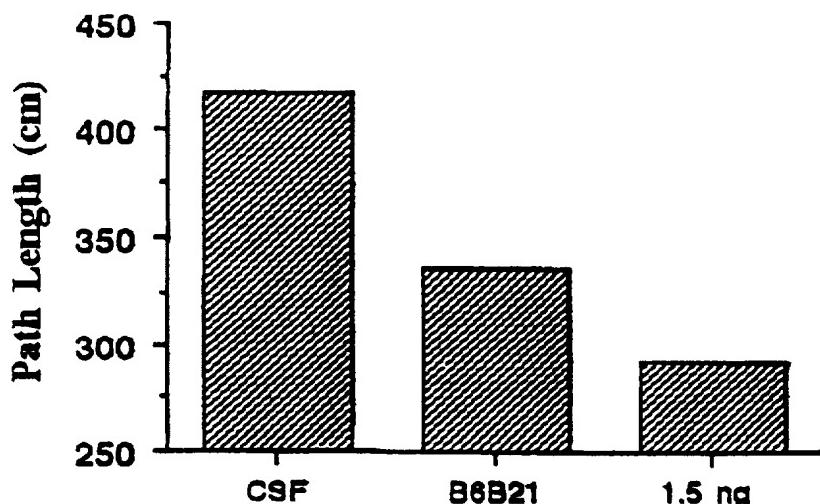


Figure 4B

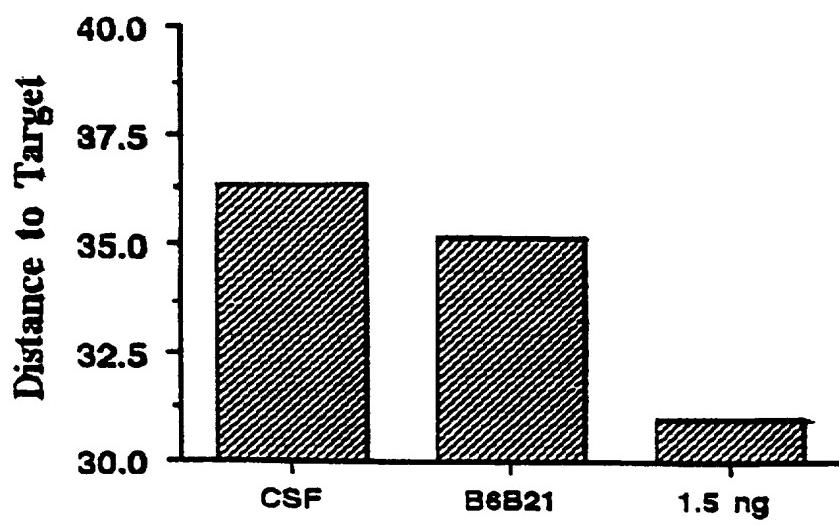


Figure 4C

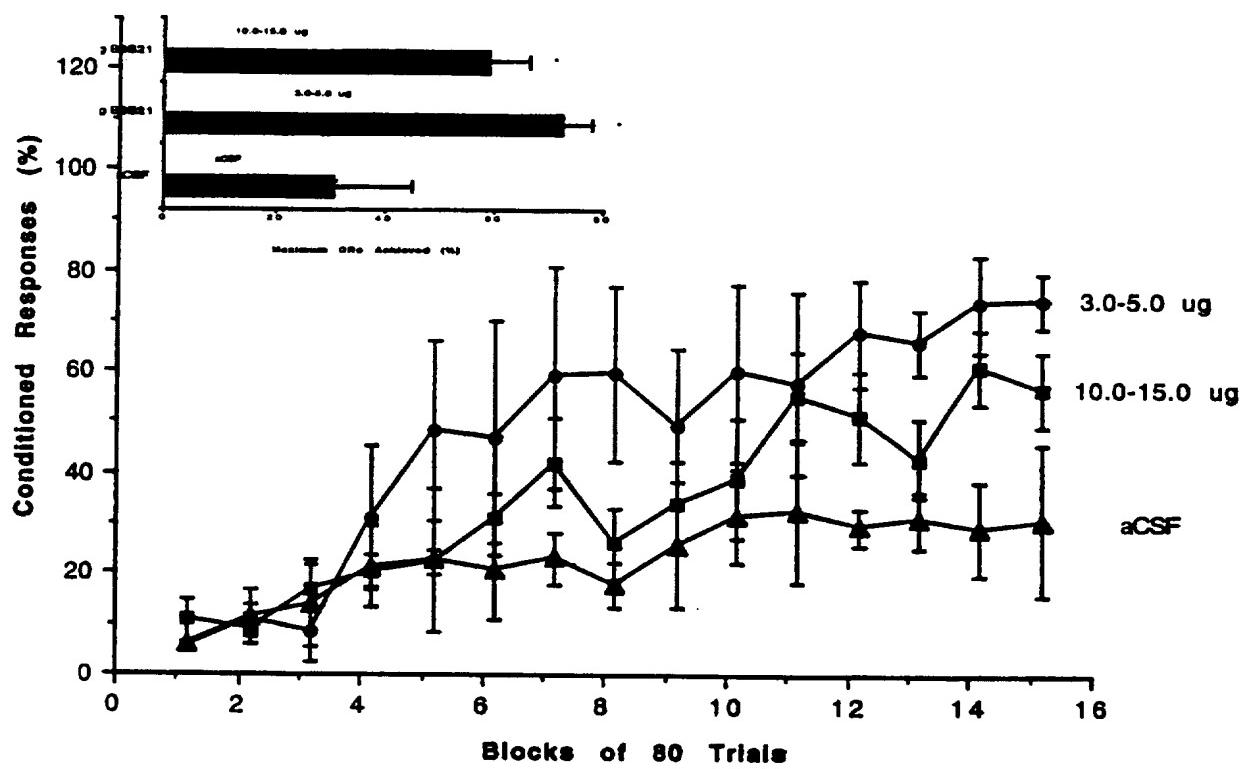
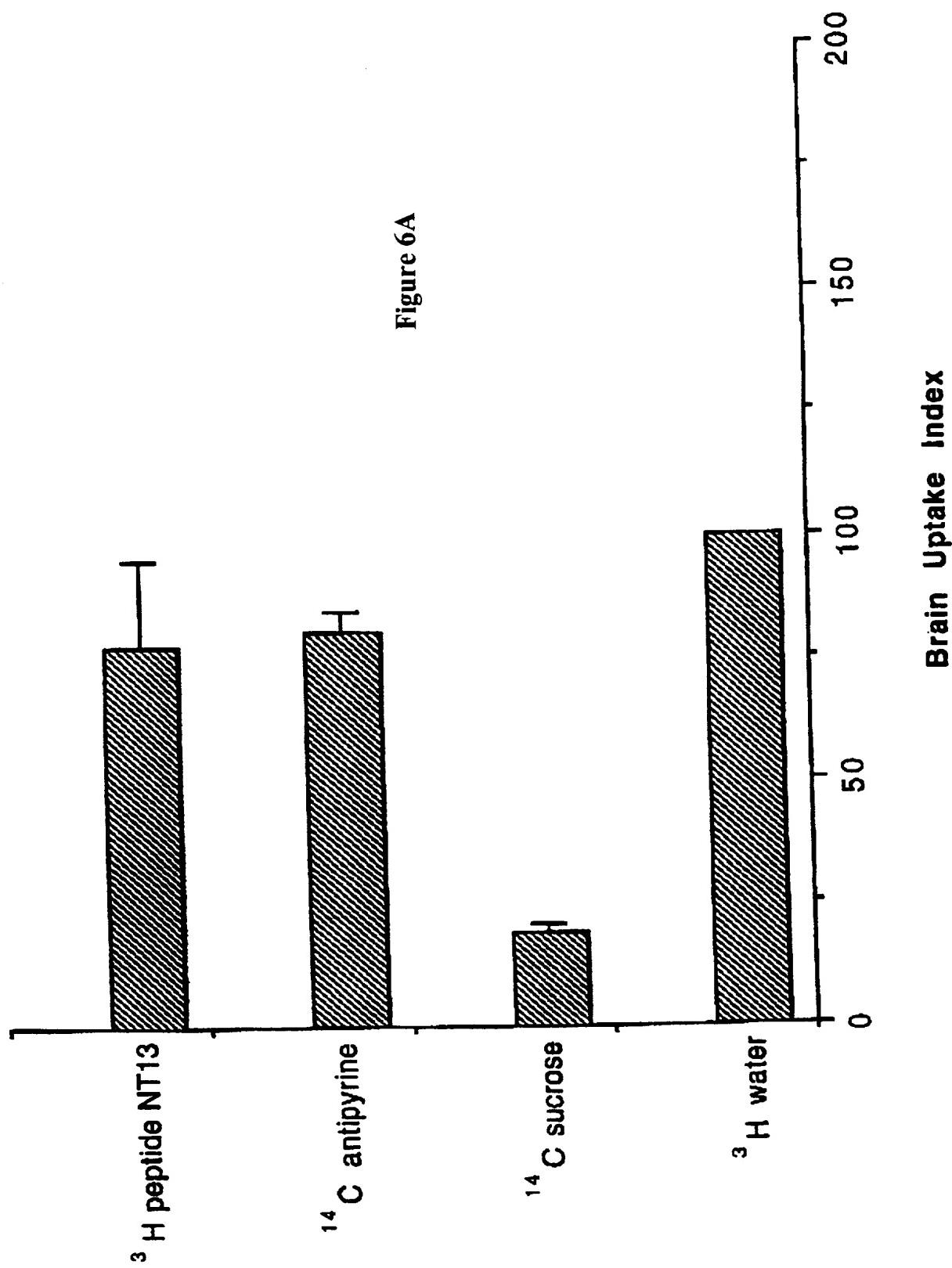


Figure 5

Figure 6A



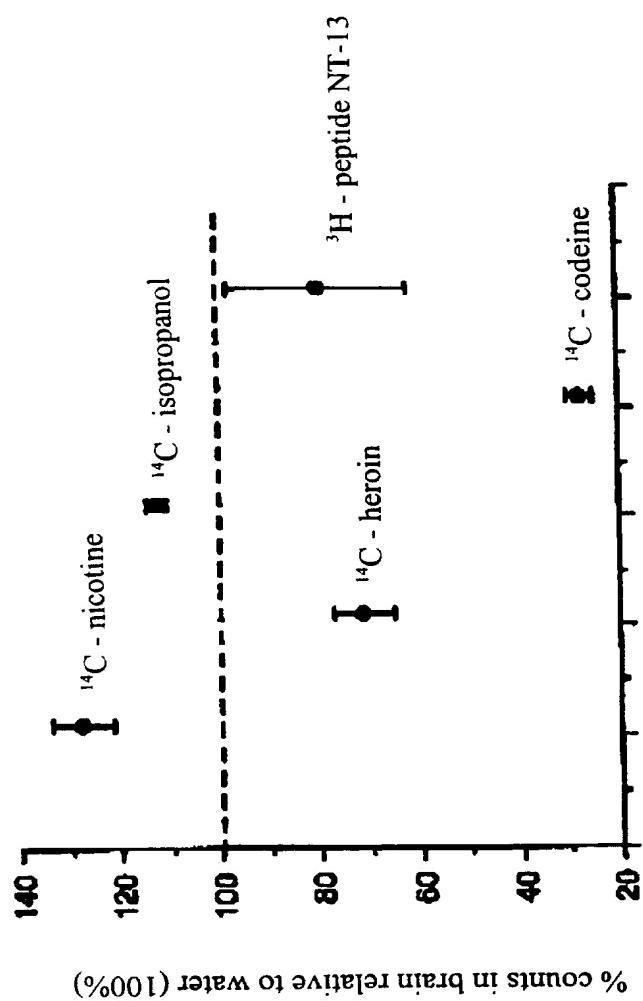


Figure 6B

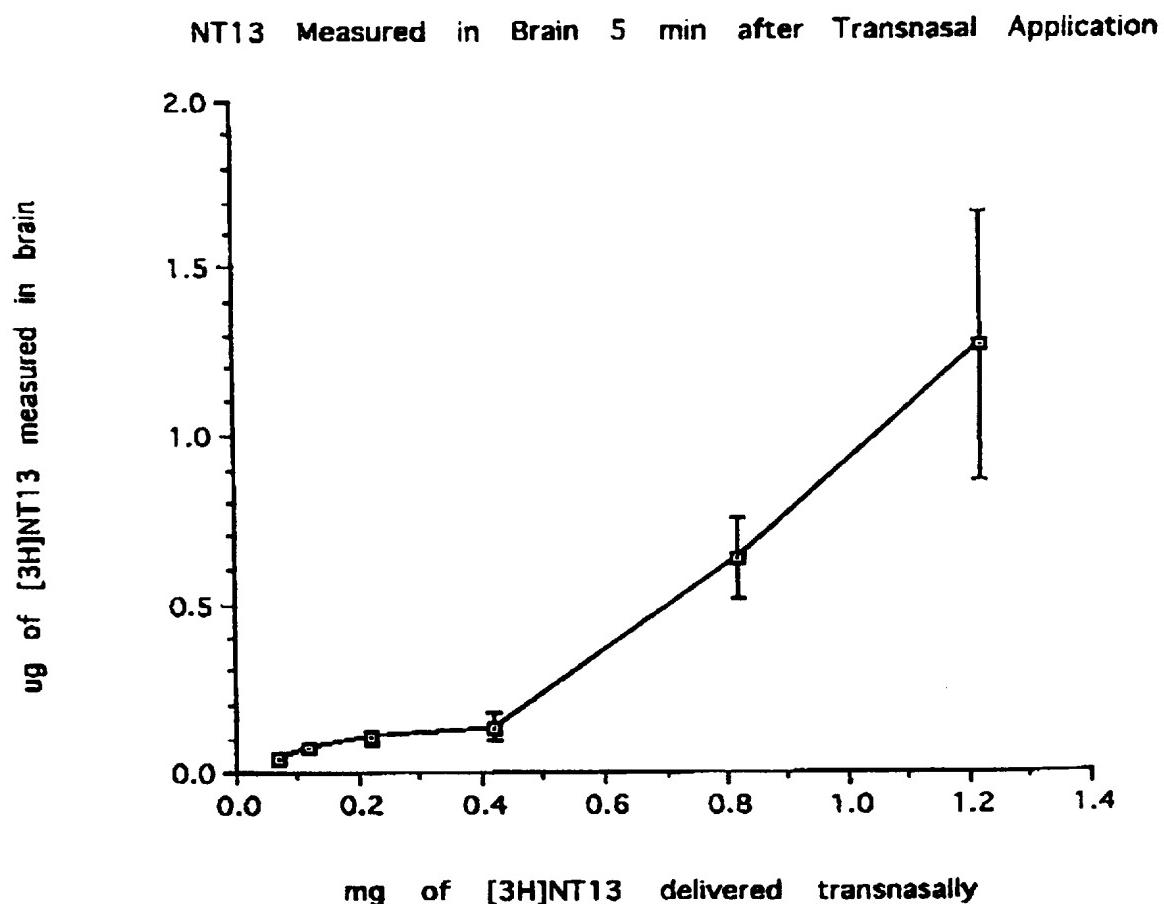
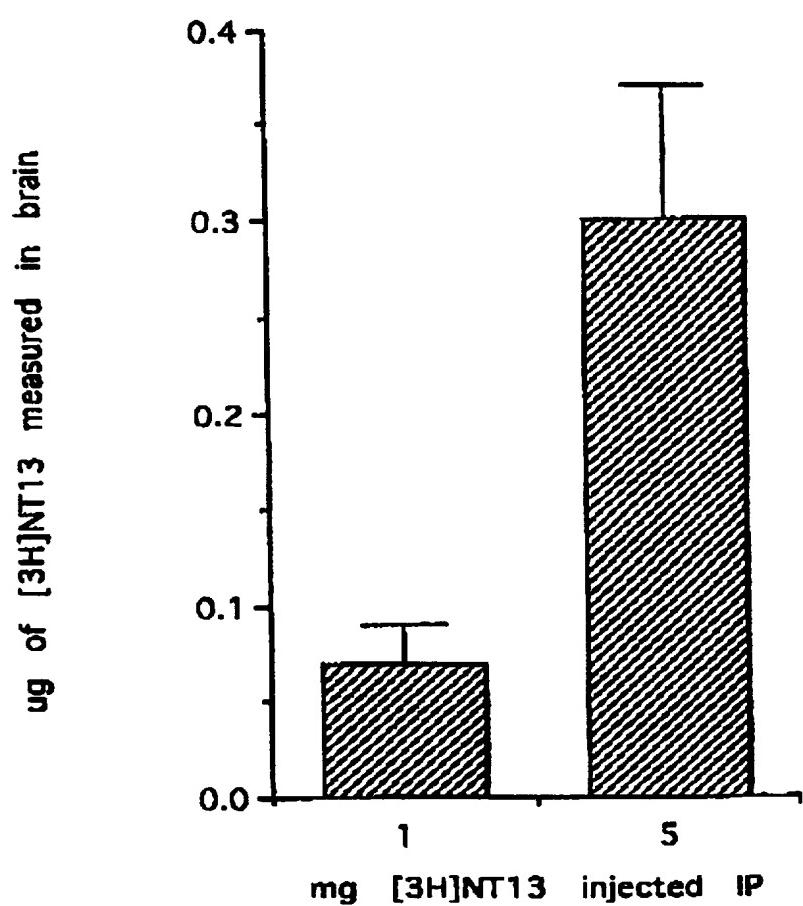


Figure 7

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NT13 Measured in Brain 20 min after IP Injection**Figure 8**

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/08667

A. CLASSIFICATION OF SUBJECT MATTER		C07K5/06	C07K5/08	C07K7/06	C07K5/103	A61K38/04
IPC 6	A61K38/16					

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 702 082 A (HAGIWARA YOSHIHIDE) 20 March 1996 whole document, especially claim 11 (2), claim 17 (2) ---	5
A	WO 96 11698 A (SYMPHONY PHARMACEUTICALS INC) 25 April 1996 whole document ---	1-5 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
17 September 1997	02.10.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Kronester-Frei, A

INTERNATIONAL SEARCH REPORT

Intern. Appl. No
PCT/US 97/08667

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE, vol. 359, 1992 - 15 October 1992, pages 638-641, XP002040919 THOMPSON L.T. ET AL.: "Hippocampus-dependent learning facilitated by a monoclonal antibody of D-cycloserine" cited in the application Abstract, pages 639 and 640, right columnspage</p> <p>-----</p>	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/08667

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0702082 A	20-03-96	JP 7101999 A US 5589573 A	18-04-95 31-12-96
WO 9611698 A	25-04-96	EP 0785793 A	30-07-97